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# The role of the serum tube agglutination test in the monitoring of human brucellosis: evaluation of post-treatment SAT titers

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## SUMMARY

**OBJECTIVE:** Positive results of the serum tube agglutination test that persist after treatment may be interpreted by clinicians as treatment failures. Therefore, our study examined the value of serum tube agglutination test in demonstrating treatment success.

**METHODS:** In this retrospective study conducted at a single center, the pre- and post-treatment serum tube agglutination test titers of patients diagnosed with brucellosis were compared.

**RESULTS:** The end-of-treatment serum tube agglutination test titer was negative in 24 (18%) of 139 patients diagnosed with brucellosis. The most common complaints of the patients were fever (78.4%), chills (88.5%), sweating (84.9%), anorexia (79.1%), and arthralgia (63.3%). The rate of positive blood culture before the treatment was 68.3%. The absence of fever (p=0.005) and arthralgia (p=0.024) and the pretreatment serum tube agglutination test titer of <1/160 (p=0.014) were significant markers of serological cure.

**CONCLUSION:** Although serum tube agglutination test is an effective and very successful test in the diagnosis of brucellosis, our study shows that serum tube agglutination test is not useful in demonstrating the treatment success of human brucellosis in the early post-treatment period.

**KEYWORDS:** Brucellosis. Bacteremia. Blood culture. Serological follow-up. Agglutination tests.

## INTRODUCTION

Brucellosis is the most common zoonotic disease worldwide and occurs mainly in areas where livestock farming is done. Despite low mortality rate, treatment failure and relapse rates are also substantial due to frequent treatment failure and repeated contacts in endemic areas[1-3].

The serum tube agglutination test (SAT) for brucellosis is mostly used for the diagnosis of this disease, and it is not recommended to use this test in treatment monitoring[4-6]. However, our daily practice shows that this test is used in the follow-up of brucellosis patients; a failure to drop titer SAT can often be considered a treatment failure in this patient population and unfortunately leads them to undergo prolonged and excessive treatments. For this reason, this study aimed to investigate the success of SAT in brucellosis monitoring by comparing pre- and post-treatment SAT titers.

## METHODS

### Study design and patients

Patients aged 16 years and older who were diagnosed with brucellosis and regularly presented to the outpatient clinic Infectious Diseases of Bitlis Tatvan State Hospital every 2–4 weeks between October 1, 2018, and December 31, 2019, were enrolled in this retrospective and single-center study. All patient data were retrieved from our previous study[7]. The study patients were also divided into two groups to compare relevant variables: the end-of-treatment SAT-positive group (those with persistent SAT positivity) and the end-of-treatment SAT-negative group (those with nonpersistent SAT positivity).

### Demographic and clinical data

The epidemiological, clinical, and laboratory findings of the patients were retrospectively evaluated. Brucellosis was diagnosed with a SAT titer of ≥1/160 or by isolation of *Brucella* spp. in a blood culture. Clinical improvement was noted in patients who received regular and appropriate treatment for at least 6 weeks and had improved symptoms and signs. A SAT titer of <1/40 at the end of the treatment was accepted as SAT negativity (serological cure), while a titer between 1/40 and 1/160 was considered a low titer. Patients who developed clinical symptoms within 6 months of the treatment and had at least a twofold increase in SAT titers or growth of *Brucella* spp. in blood culture were considered relapses.

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### Statistical analysis

The SPSS 15.0 program for Windows was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables, and median and interquartile range for numerical variables. Comparisons of numerical variables between two independent groups were performed using the Mann-Whitney U test because the condition of normal distribution was not met. Rates in groups were compared using the chi-square test. Risk factors were analyzed using the logistic regression analysis, considering variables with a $p<0.250$. The alpha significance level was set at $p<0.05$.

## RESULTS

In total, 139 patients diagnosed with brucellosis were included in the study. While 24 (18%) patients had negative end-of-treatment SAT titers, 115 (82%) had positive end-of-treatment SAT titers. Of the patients, 67 (48.2%) were male, 72 (51.8%) were female, and their mean age was 34 years (IQR 23–46) (Table 1).

In all, 29.6% of patients with persistent end-of-treatment SAT positivity and 25% of patients whose SAT became negative at the end of treatment were evaluated as having complicated brucellosis (p=0.653). The most common complaints of the patients were fever, chills, sweating, anorexia, and arthralgia. While 78% of the patients complained of fever, 52% had fever at admission. The proportion of patients with fever in the history was significantly higher (82.6 vs. 58.3%, p=0.09) in the end-of-treatment SAT-positive group (those with persistent SAT positivity) than that in the end-of-treatment SAT-negative group (those with nonpersistent SAT positivity). This difference was not observed in patients with fever at admission (51.3 vs. 54.2%, p=0.799) (Table 2).

Patients with persistent end-of-treatment SAT positivity had a significantly higher proportion of those with a pretreatment SAT titer of >1/160 (91.3 vs. 75%, p=0.034). Sixteen patients with a SAT titer of <1/160 (which is considered a low titer) were diagnosed with brucellosis after the growth of *Brucella* spp. in blood culture. In all, 2 of the 16 patients had a negative (corresponding to a SAT titer of <1/40) pretreatment SAT. Relapse occurred in five patients within 6 months of treatment. Only one of these patients was diagnosed with relapse after the SAT titer increased twofold, while three patients were diagnosed with relapse after isolation of

**Table 1.** Demographic and epidemiological characteristics of patients with brucellosis.

| Characteristics | All patients, n (%) | End-of-treatment SAT-positive group, n (%) | End-of-treatment SAT-negative group, n (%) | p |
|---|---|---|---|---|
| Number of patients | 139 | 115 | 24 | |
| Gender | | | | |
| Male | 67 (48.2) | 52 (45.2) | 15 (62.5) | 0.123 |
| Female | 72 (51.8) | 63 (54.8) | 9 (37.5) | |
| Age | 34 (23–46) | 34 (23–45) | 40.5 (27.5–48.75) | 0.301 |
| Profession | | | | |
| Livestock farming | 98 (70.5) | 78 (67.8) | 20 (83.3) | 0.130 |
| Homemaker | 16 (11.5) | 15 (13.0) | 1 (4.2) | 0.306 |
| Student | 4 (2.9) | 3 (2.6) | 1 (4.2) | 0.536 |
| Other | 21 (15.1) | 19 (16.5) | 2 (8.3) | 0.530 |
| Residence address | | | | |
| Bay | 72 (51.8) | 59 (51.3) | 13 (54.2) | 0.799 |
| District | 61 (43.9) | 50 (43.5) | 11 (45.8) | 0.833 |
| City Center | 6 (4.3) | 6 (5.2) | 0 (0.0) | 0.530 |
| Possible transmission route of the disease | | | | |
| Consumption of unpasteurized milk and milk products | 90 (64.7) | 72 (62.6) | 18 (75.0) | 0.248 |
| Contact with sick animals and their secretions | 67 (48.2) | 57 (49.6) | 10 (41.7) | 0.481 |
| Family history of brucellosis | 43 (30.9) | 34 (29.6) | 9 (37.5) | 0.444 |
| Relapse | 17 (12.2) | 14 (12.2) | 3 (12.5) | 1.000 |
| Unknown | 14 (10.1) | 12 (10.4) | 2 (8.3) | 1.000 |

*Brucella* spp. in blood culture, although SAT titers were not significant for diagnosis.

In multiple regression analysis, pretreatment SAT <160 [OR: 4.739, 95%CI 0.061–0.727, p=0.014], absence of fever [OR: 4.484, 95%CI 0.079–0.633, p=0.005], and absence of arthralgia [OR: 3.215, 95%CI 0.112–0.860, p=0.024] were found to be the predictors of post-treatment serologic cure (Table 3).

## DISCUSSION

Our study found that the end-of-treatment SAT titers became negative in only 18% of patients with brucellosis who were treated at the correct time and dose and whose clinical and laboratory results improved.

Brucella antibodies can be detected in serum for a long time after appropriate treatment[1]. In a study of 92 patients, it was

**Table 2.** Clinical characteristics of patients with brucellosis.

| Characteristics | All patients, n (%) | End-of-treatment SAT-positive group, n (%) | End-of-treatment SAT-negative group, n (%) | p |
|---|---|---|---|---|
| Clinical form | | | | |
| Acute | 104 (74.8) | 87 (75.7) | 17 (70.8) | 0.621 |
| Subacute | 35 (25.2) | 28 (24.3) | 7 (29.2) | |
| Complicated patient | | | | |
| Sacroiliitis | 12 (8.6) | 10 (8.7) | 2 (8.3) | 1.000 |
| Spondylodiscitis | 14 (10.1) | 12 (10.4) | 2 (8.3) | 1.000 |
| Peripheral arthritis | 3 (2.2) | 3 (2.6) | 0 (0.0) | 1.000 |
| Orchitis | 2 (1.5) | 1 (0.9) | 1 (4.2) | 0.321 |
| Hepatitis | 4 (2.9) | 4 (3.5) | 0 (0.0) | 1.000 |
| Other | 5 (3.6) | 4 (3.5) | 1 (4.2) | 1.000 |
| Noncomplicated patient | 99 (71.2) | 81 (70.4) | 18 (75.0) | 0.653 |
| Relapsing patient | 17 (12.2) | 14 (12.2) | 3 (12.5) | 1.000 |
| Symptoms and signs/laboratory | | | | |
| Fever (in the history) | 109 (78.4) | 95 (82.6) | 14 (58.3) | 0.009 |
| Fever (at admission) | 72 (51.8) | 59 (51.3) | 13 (54.2) | 0.799 |
| Chills | 123 (88.5) | 103 (89.6) | 20 (83.3) | 0.479 |
| Sweating | 118 (84.9) | 101 (87.8) | 17 (70.8) | 0.055 |
| Anorexia | 110 (79.1) | 91 (79.1) | 19 (79.2) | 0.997 |
| Nausea | 51 (36.7) | 39 (33.9) | 12 (50.0) | 0.137 |
| Weight loss | 15 (10.8) | 13 (11.3) | 2 (8.3) | 1.000 |
| Arthralgia | 88 (63.3) | 76 (66.1) | 12 (50.0) | 0.137 |
| Myalgia | 86 (61.9) | 69 (60.0) | 17 (70.8) | 0.320 |
| Headache | 49 (35.3) | 41 (35.7) | 8 (33.3) | 0.829 |
| Backache | 54 (38.8) | 46 (40.0) | 8 (33.3) | 0.542 |
| Splenomegaly | 49 (35.3) | 43 (37.4) | 6 (25.0) | 0.248 |
| Hepatomegaly | 36 (25.9) | 33 (28.7) | 3 (12.5) | 0.099 |
| Lymphadenomegaly | 10 (7.2) | 8 (7.0) | 2 (8.3) | 0.683 |
| Anemia (Hgb g/dl) (<12 for females, <14 for males) | 53 (38.1) | 44 (38.3) | 9 (37.5) | 0.944 |
| Leukopenia (<4000/mm$^3$) | 10 (7.2) | 9 (7.8) | 1 (4.2) | 1.000 |
| Thrombocytopenia (<150,000/mm$^3$) | 12 (8.6) | 11 (9.6) | 1 (4.2) | 0.691 |
| Positive blood culture | 95 (68.3) | 81 (70.4) | 14 (58.3) | 0.246 |
| Prognosis | | | | |
| Clinical improvement | 134 (96.4) | 110 (95.7) | 24 (100) | 0.587 |

Bold value indicates statistical significance at the p<0.05 level.

**Table 3.** Factors determining post-treatment SAT negativity in patients with brucellosis.

| | p | OR | 95%CI | |
|---|---|---|---|---|
| Gender (ref: male) Female | 0.082 | 2.392 | 0.156 | 1.118 |
| Absence of fever (in the history) | 0.005 | 4.484 | 0.079 | 0.633 |
| Absence of arthralgia | 0.024 | 3.215 | 0.112 | 0.860 |
| Pretreatment SAT titer <1/160 | 0.014 | 4.739 | 0.061 | 0.727 |

Bold values indicate statistical significance at the p<0.05 level.

reported that SAT titers were still positive in 48% of patients 1.5 years after the completion of treatment[8]. In another study comparing pre- and post-treatment SAT titers, it was found that SAT positivity persisted 80% after treatment and the 2 ME (mercaptoethanol) agglutination test became negative in all patients[9]. Similarly, the end-of-treatment SAT positivity rate was 82% in our study. In a study in which the SAT titers of 175 patients with brucellosis who showed clinical improvement were followed for 2 years, serological cure was 24% at 1 year and 87% at 2 years[10]. In a similar study conducted in patients with acute brucellosis in Saudi Arabia, where brucellosis is endemic, the SAT cure rate in the 1st month after treatment was reported to be 8.3%, and in the same study, male gender, advanced age, and use of fewer than three antibiotics during treatment were associated with post-treatment SAT positivity in the univariate analysis. At the same time, no significant marker of serological cure was found in the multiple analysis[11]. According to a report from another endemic region of our country, the post-treatment serological cure was 29.3%[12]. However, in our study, the post-treatment serological cure was 18%, and when multiple analyses were performed, a pretreatment SAT titer of <1/160 (OR: 4.739), absence of fever (OR: 4.484), and absence of arthralgia (OR: 3.215) were found to be important predictors for serological cure (Table 3).

In a study in which the SAT titers of 35 patients diagnosed with and recovered from brucellosis were followed for 2 years, it was shown that this test became negative to varying degrees and with low frequency in persistent and relapsing patients, whereas it became negative more rapidly and to a greater extent in patients with acute brucellosis. The same study concluded that the SAT is not suitable for monitoring chronic patients and predicting relapses[6]. In our study, the proportion of patients with acute disease and relapse was similar in the end-of-treatment SAT-positive and SAT-negative groups (75.7 and 70.8%, p=0.621; 12.2 and 12.5%, p=1, respectively) (Table 2). Our study also measured SAT titers only in the 1st month after treatment, and SAT titers decreased in 59% of patients and remained the same in 23%. Of our five relapsing patients, only one was diagnosed with relapse after SAT titers increased two-fold, while three were diagnosed with relapse after isolation of *Brucella* spp. in blood culture, although SAT titers were not significant for diagnosis. Our data support studies showing that SAT is not very successful in detecting relapse.

When comparing Brucella isolation rates before treatment, no statistical difference was found between patients with the end-of-treatment SAT-positive and SAT-negative (70.4 vs. 58.3%, p=0.246). Since bacteremia can occur periodically in brucellosis, blood culture is not always useful to demonstrate treatment success and clinical improvement[13]. In contrast, culture tests are not useful in monitoring brucellosis because of the high risk of contamination via the laboratory[14]. In our study, 16 patients with a SAT titer of <1/160 were diagnosed with brucellosis based on the isolation of *Brucella* spp. in blood culture. A blood culture may be a suitable diagnostic tool to diagnose early acute brucellosis when serological tests are not yet positive or to diagnose relapse when serological tests are inadequate and unreliable[9,15,16].

Coactivation of the humoral and cellular immune systems is one of the defense mechanisms developed by the host against *Brucella* bacteria. Cellular immunity plays the main role in healing. Although the presence of specific antibodies is important in diagnosing the disease, their success in monitoring the disease is limited. After successful treatment, IgM-type antibodies may be positive in low titers for months or years[1]. The long-term presence of *Brucella* antibodies makes it difficult to distinguish from relapse or previous infection. In this case, high IgG avidity is helpful to rule out active infection[1,17].

Although enzyme immunoassay (EIA) is not superior to other serological tests in the diagnosis of brucellosis, it is the most sensitive serological test in disease surveillance[18,19]. A study investigating the value of 2-ME and *Brucella* EIA in treatment monitoring revealed that IgM (EIA) is safer in the diagnosis and treatment monitoring of acute brucellosis, preventing 45.6% of unnecessary treatments[20]. In their study, Tumturk et al. demonstrated a significant difference between pre- and post-treatment IgM titers in patients with brucellosis and reported that there was no such difference between pre- and post-treatment IgG levels, especially in chronic patients. Based on these data, they concluded that the combined use of IgG and IgM tests in the diagnosis and monitoring of brucellosis would provide more accurate results[21].

When brucellosis is not treated, chronic infections and relapses may occur, which are undesirable complications of brucellosis. The evaluation of serological tests in conjunction

with clinical characteristics may be helpful to assess the success of treatment. EIA, 2-ME, Brucellacapt, and Coombs tests are now available for serological monitoring. Tests other than the EIA are relatively inexpensive and can be easily performed at any center. Rational serological surveillance with clinical data can prevent unnecessary antibiotic treatment and make a positive contribution to patient and environmental health.

Considering the data in the literature and in our study, it has been elucidated that SAT is not suitable to monitor brucellosis and to indicate the success of brucellosis treatment, apart from its high success in diagnosing the disease. However, clinicians may have to use this test for treatment follow-up. In this case, the interpretation of SAT by experienced physicians and/or combined use with other antibody tests may prevent prolonged treatments and increased treatment costs. Therefore, there is a need for tests that can be used to monitor the disease and are easy to interpret so that persistent SAT titer positivity can result in unnecessary and prolonged treatment, but there is still a need for comprehensive studies on this topic.

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All procedures performed in this study involving human participants were in accordance with the ethical standards of the National Research Committee and with the ethical standards of the Declaration of Helsinki. This study was approved by the Ethics Committee of Haseki Training and Research Hospital (approval number: 2021-67, date: 14/07/2021). Written informed consent was waived, given the retrospective nature of this study.

## AUTHORS' CONTRIBUTIONS

**BC**: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **OP**: Conceptualization, Data curation, Visualization.

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